RESEARCH REPORT
PORK SAFETY

Title: *Salmonella* serovar distribution and persistence in finisher sites – **NPB #12-069**

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Industry Summary: There is a paucity of data explaining the persistence of *Salmonella* serovars on swine farms. In this study, we evaluated 900 pigs from 18 cohorts of finisher swine over a 3.5 year period in one production company to understand the distribution and persistence of *Salmonella* serovars on swine farms and to identify risk factors associated with persistence of *Salmonella* serovars in swine. Based on the results of this study, the duration of *Salmonella* shedding in pigs is associated with the *Salmonella* status of the nursery of origin (more positive samples from the nursery, the longer the duration of shedding), infection at a younger age (pigs first detected as positive at 10 weeks of age shed *Salmonella* longer than pigs 12 week of age or older at first positive *Salmonella* fecal sample) *Salmonella* serovar (*S.* Agona was shed for a longer duration) and the number of treatments in the group of pigs from which the pig originates (above average treatment rate decreased the duration of *Salmonella* shedding in swine).

Keywords: *Salmonella*, serovar, persistence, pork safety

Scientific Abstract:

The objective of this study was to describe the *Salmonella* servoar distribution on swine farms, compare persistence of different serovars in finishing swine and to identify management factors associated with *Salmonella* servoar persistence in swine. A study was carried out in one swine production system representing 900 pigs from 18 cohorts of finishing swine. The six most common serovars isolated from this farm were *S. enterica* serovar Derby (47.3%), *S.* Agona (27.4%), *S.* Johannesburg (10.5%), *S.* Schwarzengrund (2.7%), *S.* Litchfield (2.5%) and *S.* Mbandaka (2.2%). Pigs detected *Salmonella* positive for the first time at 10 weeks of age had a longer duration of shedding, than pigs first detected positive at an older age. The duration of shedding was shorter among pigs infected with *S.* Derby, *S.* Johannesburg and other serovars as compared to pigs infected with *S.* Agona. A significant difference was observed among sites despite belonging to the same production company. Cohorts with pig treatment proportions greater than the mean were more likely to have a shorter duration of *Salmonella* shedding. There was a significant influence of nursery status on duration of fecal shedding. Pigs from cohorts with nursery positive pools greater than the overall mean had a longer duration of *Salmonella* shedding as compared to pigs from cohorts with nursery pools less than or equal to the mean.

These results suggest that the duration of *Salmonella* shedding may depend on Salmonella serovar, pig age at infection, farm site and cohort level risk factors. Identification of risk factors associated with the duration of shedding may allow more targeted interventions for the control Salmonella by evaluation of control measures not only for prevalence reduction, but also to decrease the duration of shedding, which may decrease the risk of contamination of pork and subsequent risk of foodborne illness.

Introduction:

Salmonellosis remains a major foodborne disease threat to public health in the United States. Although significant strides have been made in reducing the incidence of bacterial foodborne illnesses (*Camplylobacter, Yersinia enterocolitica* and Shiga Toxigenic *Escherichia coli*) with reductions of ~20-50% relative to 1996-1998 rates, incidence of human salmonellosis has had at most a modest decrease in that same period. Clearly, there is a need to further pursue effective interventions for salmonellosis.

Salmonella serovars have been recognized to have differential ability to cause illness in different species (e.g. host-adapted serovars such as Choleraesuis in swine, Typhi in humans and Gallinarum in poultry). There is further evidence that some serovars, although capable of zoonotic transmission, are more commonly associated with certain food animal species as compared to others (e.g Derby in swine). In fact, this serovar distribution by species has been utilized for purposes of determining foodborne disease attribution ultimately to determine where efforts for control of human foodborne disease can be most efficiently focused.

One area of limited knowledge is the epidemiology of *Salmonella* serovars. Few on-farm studies have evaluated *Salmonella* serovars based on their distribution both within and across production systems, as well as a paucity of effort to evaluate whether there are risk factors associated with persistence of certain serovars in swine production systems. Knowing these risk factors may allow more targeted interventions to control those serovars that are more commonly associated with foodborne transmission, ultimately resulting in on-farm interventions that are more efficiently targeted at control of *Salmonella* serovars likely to result in human illness.

Objectives:

1. Describe *Salmonella* serovar distribution in growing swine.

The need addressed by this objective is to describe the serovar distribution across sites, within cohorts and over time in a swine production system. The approach is a prospective cohort study of *Salmonella* serovars isolated from growing swine in one swine production company (18 cohorts of swine from 3 finisher sites (3 sites x 6 cohorts/site) conducted over a 3.5 year period.

2. Compare the persistence of *Salmonella* serovars.

The working hypothesis is that different *Salmonella* serovars will have differential persistence in swine and swine environments. The approach is a prospective cohort study comparing the duration of shedding between *Salmonella* serovars isolated during the finishing phase.

3. Identify management factors associated with serovar persistence.

The hypothesis is that there will be management factors that will be associated with persistence of certain *Salmonella* serovars compared to others. The approach is a prospective cohort study evaluating the association between management factors and serovar persistence in growing swine.

Materials & Methods:

The samples were collected from a longitudinal study of growing pigs in a multi-site farrow-to-finish production system (3 finishing sites with 6 cohorts each, 18 cohorts total) located in the Midwestern, United States (Pires et al., 2013a). Selection criteria, description of the production system, finishing sites, study design, sampling strategy were published elsewhere (Pires et al, 2013a; Pires, 2013b). Fifty individual pig fecal samples per cohort were collected and cultured using standard methods every 2 weeks for 16 weeks (18 cohorts X 50 pigs/cohort x 8 collections =900) (Pires et al., 2013a). All 187 *Salmonella*-positive pigs (identified as a pig that had at least 1 Salmonella positive fecal sample; 187 pigs/x pigs total) were included in the current study. The total isolates included in this study were: 446 isolates from individual pig samples, 63 isolates from pooled fecal samples from source nurseries and 33 isolates from environment samples taken from the finisher barn after cleaning and disinfection and prior to pig placement. Identification of serotypes was conducted according to the Kauffmann-White scheme and was conducted by MSU Diagnostic Center for Population and Animal Health and the National Veterinary Services Laboratory, Ames, IA..

Description of the risk factors

Risk factors investigated in this study were: sex (male/female); pig heath status; pig age (weeks) at the first *Salmonella* positive sample; site (A, B and C); nursery and environment *Salmonella* status; proportion of individual treatments and proportion of "at risk pig" in each respective cohort (Pires et al., 2013b); presence of the same serovar in nursery or environment samples and respective cohort (defined from now on as serovar exposure, Yes or No). A pig was considered healthy if none of the following events occurred at the sampling time: 1) diarrhea; 2) sick or being moved to the sick pen; 3) undersized pig; 4) subject pig ("at risk pig", definition below); 5) any sign of disease observed by research team personnel (e.g., lameness, diarrhea, respiratory signs). Pigs were classified as "at risk pig" if a pig appeared abnormal for any reason, tagged with a unique tag and housed separately. The nursery *Salmonella* status variable was categorized into 2 levels based on the overall mean of the total positive pools (reference level: less than or equal to the mean, 3.64%). The barn environmental status variable was categorized as positive when at least one sample was *Salmonella* positive (reference level: all swabs were negative). The proportion of "at risk pig" and treatment (estimated by total number of individual treatments) were estimated as a proportion based on total of pigs placed at the beginning of each cohort and then categorized in 2 levels depending on central tendency; less than or equal to the mean (1.88%, % "at risk pig" reference level) or median (0.48%, % treatments reference level), and greater than the median for all cohorts.

Statistical analyses, model building and residual analysis

Data analysis was performed using the software Stata version11.0 (StataCorp LP College Station, TX). The relative proportion of serovars for each sample type (individual pig fecal sample, pooled fecal sample from nursery and environmental swabs) was described. To determine measures of serovar persistence, time to event data (days of shedding) was calculated. For individual pigs, the duration of shedding for each new infection was defined as the interval between the sampling date of the first *Salmonella*-positive culture and the sampling date of the last positive culture for an individual pig; 7 days were added to the shedding duration to account for shedding during the sampling interval (2 week sampling intervals) after the last positive culture. Two datasets were built for survival analysis in order to investigate the risk factors associated with *Salmonella* persistence in general (dataset 1) and serovar-specific persistence (dataset 2) during the study period. *Salmonella*-positive pigs were included in survival analysis (data set 1 and 2) based on the following inclusion criteria: 1) survival until marketing; 2) no more than one period from which a sample was not collected; and 3) had no more than two negative cultures between *Salmonella*-positive collections. The total of *Salmonella*-positive pigs in dataset 1 was 175 pigs. In dataset 2, in addition to the described inclusion criteria, only pigs detected as shedding one unique serovar during the study period were included (total 151 pigs).

Preliminary univariable analysis was conducted using non-parametric Kaplan-Meier survival methods, in order to visually assess the survival curves (by risk factor groups and four major serovars, dataset 1). Differences in survivor functions across groups (site, sex, nursery and environment *Salmonella* status, nursery and environment serovar exposure, pig health status, pig age, treatment and "at risk pig" variables) were initially evaluated using the Log-rank test. Estimation of shedding duration was determined for the 4 major serovars in the 151 pigs shedding one unique serovar (dataset 2). Pigs shedding one unique serovar were classified in 4 groups: 3 major ones (*S.* Derby; *S.* Agona, and *S.* Johannesburg) and all other serovars, these groups were used to estimate the differences on shedding using Log-Rank test and in multivariable model (dataset 2).

Acceleration failure time (AFT) models, with log-normal distribution were used to analyze the effect of the risk factors on duration of *Salmonella* shedding (dataset 1) and *Salmonella* shedding in pigs shedding one unique serovar (dataset 2). The selection of the type of model and distribution (log-normal, log-logistic, exponential, Weibull) was based on visualization of the baseline hazard curve from the Cox and the piece-wise exponential models, and based on the lowest Akaike information criterion (AIC) for non-nested models and likelihood-ratio test (LRT) for nested models (Cleves et al., 2010b, a). The baseline hazard curve of the Cox and piece-wise exponential models had a non-monotone shape, in addition the log-normal distribution had the lowest AIC; therefore, acceleration failure time (AFT) models, with log-normal distribution were the best fit for the data.

In addition, in order to evaluate the presence of heterogeneity among cohorts, shared frailty models (Weibull and log-normal with gamma-shared frailty) and Poisson approach (with random effects) were analyzed (Rabe-Hesketh and Skrondal, 2012). The selection criteria were based on magnitude of the frailty parameter and LRT, which tests the null hypothesis: H_0: the frailty variance (theta, estimate of the variance between cohorts) is equal to zero (Rabe-Hesketh and Skrondal, 2012). No evidence of heterogeneity among cohorts was found, the frailty effect was found insignificant. A conservative approach was taken, and possible dependence among outcome events within the same cohort was taken into account by using robust standard errors of estimates. Backward elimination was conducted; selection of the variables was based on the LRT of nested models and Wald test at a significant level of 0.05. First-order interactions with biological importance and confounders were evaluated. A change of more than 20% on any of the estimates in the final model after the re-introduction of a risk factor was considered a confounding effect. The assumption of independent censoring was evaluated by sensitivity analysis comparing scenarios with changed positive and negative correlations between censoring and new infection events. Model diagnostics for the AFT models were based on overall goodness-of-fit by plotting the Cox-Snell residuals and identification of outliers by plotting deviance residuals by time; and influential points by plots of score residuals by time.

The event of interest was *Salmonella*-negative (clearance of *Salmonella*), thus survival time was interpreted as the time period (days) a pig shed *Salmonella*. The survival models are expressed in accelerated failure time metrics, in other words, positive coefficients refer to longer lengths of shedding. The time ratio was calculated for significant variables. The time ratio is interpreted as follows: if the ratio is greater than 1, the time passes more slowly for the subject (the time is decelerated) so failure would be expected occur later (the duration *Salmonella* shedding is longer than average); if it is less than 1, the time is accelerated, so the time passes more quickly for the subject and the failure would be expected occur sooner (*Salmonella* shedding times are shorter than average)

Salmonella PFGE Methods:

A total of 104 isolates (89 pig isolates, 14 nursery isolates, 4 environment isolates) were submitted to PFGE . The isolates were systematically selected in order to investigate spatial and temporal variation between sites, within cohort and between cohort and respective nursery and barn environment. The following criteria were taken into account: 1) the top three serovars: S. Derby, S. Agona and S. Johannesburg; 2) the same serovar was selected within site, within cohort and between sites; 3) the same present in the nursery or environment and

respective cohort4) three additional serovars S. Meleagridis, S. Litchfield and S. Infantis were selected to include as being present in the finisher barn after cleaning and disinfection and prior to pig placement. Standard PFGE methodology was conducted by the MSU Diagnostic Center for Population and Animal Health

Results: Report your research results by objective.

1. Describe *Salmonella* serovar distribution in growing swine.

Among the 446 Salmonella isolates (187 pigs total) 18 distinct serovars were identified. The six most common serovars were S. Derby (47.3%), S. Agona (27.4%), S. Johannesburg (10.5%), S. Schwarzengrund (2.7%), S. Litchfield (2.5%) and S. Mbandaka (2.2%) (Table1). In 12 cohorts (70.6%; 12/17) *Salmonella* serovars isolated in nursery were also isolated from individual pigs during the finishing period. In 5 of 18 (27.8%) of cohorts serovars isolated in the barn environment were also isolated from individual pigs (Table 1). In site A, 2 to 6 distinct serovars were identified per cohort; site B, 1 to 6 and site C, 1 to 4 distinct serovars were isolated. Site A had a total of 13 serovar types isolated, site B and C each had 8 different serovars isolated. The distribution of observations (number of events) stratified by site, pig health status, sex, pig age, *Salmonella* nursery status, environment *Salmonella* status; nursery and environment serovar exposure, proportion of treatments, proportion of subject pigs ("at risk pig"), is presented in Table 2, for 175 finishing pigs (dataset 1) which satisfied the inclusion criteria.

The analysis of the first dataset (all *Salmonella*-positive pigs) included 175 pigs and 151 events, of which 24 observations (pigs) were right-censored (13.7%). Overall, the Kaplan-Meier median duration of fecal *Salmonella* shedding was 28 days, and the maximum 112 days. The median duration of shedding for pigs (shedding one unique serovar colonized with *S.* Derby was 28 days and 14 days for pigs colonized with *S.* Agona, *S.* Johannesburg and *S.* Schwarzengrund. There was a significant difference in duration of shedding among sites (Log-rank test statistic 24.49, $p<0.001$); between nursery *Salmonella* statuses (Log-rank test statistic 10.83, $p=0.001$) and nursery serovar exposure (Log-rank test statistic 5.96, $p=0.015$); environment *Salmonella* status (Log-rank test statistic 11.67, $p<0.001$), categories based on the proportion of pigs treated (Log-rank test statistic 6.12, $p=0.013$), and categories based on the proportion of "at risk pig" (Log-rank test statistic 6.12, $p=0.013$) and age at first positive sample (Log-rank test statistic 12.34, $p <0.001$). In Fig. 1 is illustrated the Kaplan-Meier graphs of survivorship function for two cohort risk factors: proportion of pigs treated and nursery *Salmonella* status. No significant difference was found between sexes (Log-rank test statistic 0.99, $p=0.32$), pig health status (Log-rank test statistic 1.36, $p=0.25$); serovar exposure in the environment (Log-rank test statistic 2.24, $p=0.13$); or serovar group 4 groups: *S.* Derby, *S.* Agona, *S.* Johannesburg and all other serovars, among those pigs shedding one unique serovar (dataset 2) (Log-rank test statistic 2.54, $p=0.47$).

Objectives 2&3: Compare the persistence and risk factors for persistence of *Salmonella* serovars.

Results of AFT model for *Salmonella* persistence (dataset 1)

In this analysis 175 pigs were included, a total of 151 events of events were observed, of which 24 observations (pigs) were right-censored (13.7%). The significant explanatory variables/ risk factors for the AFT model are presented in Table 3. The time ratio was calculated for significant variables. There was a significant effect of pig age on duration of shedding, the median survival time was 59% longer (time ratio=1.59; 95% C.I. 1.13- 2.22) for pigs that were detected *Salmonella* positive for the first time at 10 weeks of age as compared to pigs for which their first positive fecal sampled occurred at 12 weeks or older. There was a significant difference in the duration of *Salmonella* shedding among pigs from cohorts with different nursery status. The median survival time was 70% longer (time ratio=1.7; 95% C.I. 1.33- 2.17) for pigs from cohorts with nursery positive pools greater than the overall mean as compared to pigs from cohorts with nursery pools less than or

equal to the mean. There was a significant interaction between site and cohort treatment proportion in duration of shedding. Pigs that were from cohorts with treatment proportion greater than the median, the median survival time was shorter in site A (time ratio=0.71; 95% C.I. 0.56- 0.9) and site B (time ratio=0.42; 95% C.I. 0.32- 0.57) as compared to pigs from cohorts in site C with a proportion of treatment less than or equal to the median. No significant influence of the sex, pig health status, "at pig risk" proportion, environment *Salmonella* status, environment or nursery serovar exposures were found. The cumulative hazard of the Cox-Snell residuals plot follows a straight 45° line, except for 8 pigs with Cox-Snell residuals greater than 2.5. The plots of deviance and score residuals showed a few pigs with large residuals/outliers. A sensitivity analysis was conducted by deleting those observations and no significant change of the magnitude of the estimates of the coefficients was found. Therefore all pigs were included in the final analysis.

Results of AFT model for the *Salmonella* serovar-specific persistence (dataset 2)

In this analysis 151 pigs met the inclusion criteria and a total 129 of events were observed, of which 22 observations (pigs) were right censored (14.5%). The significant explanatory variables/ risk factors for the AFT model are presented in Table 4. No significant interactions were found.
For pigs that were detected *Salmonella* positive for the first time at 10 weeks of age, the median survival time was 60% longer (time ratio=1.6; 95% C.I. 1.2- 2.1) as compared to pigs for which their first positive fecal sampled occurred at 12 weeks or older. For pigs from cohorts with treatment proportion greater than the median, the median survival time was 60% shorter (time ratio=0.6; 95% C.I. 0.5- 0.8) as compared to pigs from cohorts with treatment proportion less than or equal to the mean. There was a significant effect of site in duration of shedding, the median survival time was longer in site A (time ratio=1.5; 95% C.I. 1.04-2.1) and site B (time ratio=2; 95% C.I. 1.3-3) as compared to site C. There was a significant difference in the duration of *Salmonella* shedding among serovars, the median survival time was shorter among pigs infected with *S.* Derby (56%), *S.* Johannesburg (49%) and the all other serovar group (72%) as compared to pigs infected with *S.* Agona. In Fig. 2 is illustrated the estimated survival probability plots of time in days for *Salmonella* shedding infected with one unique serovar, stratified by age and serovar. There was no significant influence of the sex, pig health status, "at risk pig" proportion, environment and nursery *Salmonella* status; nor the presence of a common serovar in environment or nursery in this model. The sensitivity analysis of the outliers was performed as described before. The cumulative hazard of the Cox-Snell residuals plot follows a straight 45° line, except for 8 pigs with Cox-Snell residuals greater than 2.5. The plots of deviance and score residuals showed a few pigs with large residuals/outliers. A sensitivity analysis was conducted by deleting those observations and no significant change of the magnitude of the estimates of the coefficients was found. Therefore all pigs were included in the final analysis.

PFGE Results

A total of 104 isolates (89 pig isolates, 14 nursery isolates, 4 environment isolates) were submitted to PFGE. Forty-five distinguishable PFGE patterns were identified (Table 1). The 3 serovars with higher number of distinct patters were: *S.* Derby (16), *S.* Agona (13) and *S.* Johannesburg (10) (Table 5).

Discussion: Explain your research results and include a summary of the results that is of immediate or future benefit to pork producers.

A number of studies have described Salmonella prevalence and serovar distribution, mainly related to risk factor studies in finishing pigs (Funk et al., 2001; Gebreyes et al., 2004; Rajic et al., 2005); but few have examined the duration of shedding and potential risk factors associated with shedding duration. The four most common serovars found in this study have been reported in other studies in North America (Funk et al., 2001; Gebreyes et al., 2004; Rajic et al., 2005; Keelara et al., 2013). The majority of the isolated serovars (e.g., S. Derby, S. Agona and S. Johannesburg) have been

associated with human illness (Jones et al., 2008; CDC, 2013b) and related to pork consumption (Hauser et al., 2011; CDC, 2013b). This study indentified an effect of serovar on the duration of Salmonella shedding which it might be used for future mitigation measures on decreasing potential carcass contamination at slaughter and ultimately, human disease ; in particular for those serovars that represent a higher proportion of invasive disease in humans (e.g., S. Choleraesuis and S. Typhimurium) (Jones et al., 2008).

Pigs detected as Salmonella positive at 10 weeks of age had a longer duration of shedding in this study. This is the first report we are aware of indicating a differential risk for the duration of shedding based on age of first detection in pigs. It may be a cause for the common report of decreasing prevalence over the duration of the finishing period in swine (Kranker et al., 2003; Nollet et al., 2005; Vigo et al., 2009; Molla et al., 2010). Although a novel finding, several biologically plausible reasons may explain this result. Young pigs are more susceptible to infections (Carroll et al., 2001; Sutherland et al., 2005); therefore, the longer duration of shedding may be associated with a higher susceptibility to infection in earlier age and/or higher infectiveness and ability of the pathogen to establish a permanent infection in earlier age stages. Experimental studies have shown a decrease in Salmonella shedding (both based on prevalence and concentration shed) after challenge, independent of the serovar. However the age of those pigs were the same within each study, with all being infected at 6 or 10 weeks of age, precluding evaluation of the effect of pig age (Osterberg and Wallgren, 2008; Scherer et al., 2008; Osterberg et al., 2009). Another potential explanation is the data structure itself. The data were dichotomized in this study (10 weeks of age or > 10 weeks of age) as a result of the distribution of Salmonella positive fecal samples (Pires et al., 2013a). Very few pigs were detected positive for the first time after 10 weeks of age. Therefore whether it is a function of age or another aspect of this dataset is unclear (time of exposure, specific risks for this production company, etc). Despite this limitation, further evaluation of the association of age of Salmonella exposure and duration of shedding is warranted, as it may assist in identifying efficacious interventions, as well as age specific targeting of interventions to decrease Salmonella shedding in swine.

To our knowledge no other studies have been published regarding Salmonella fecal shedding persistence in swine using survival methods. Despite the finding that pigs infected with S. Derby had the longest shedding period in the Kaplan-Meier analysis, the survival time was shorter among pigs infected with S. Derby, S. Johannesburg and others serovars as compared to pigs infected with S. Agona in the final multivariable model. The differential duration of shedding of serovars might lead a higher risk to transmission among pigs and longer persistence of the serovar in the farm. Furthermore, it may increase the public health risk of those serovars being introduced in food chain; S. Agona was among the 20 most frequently reported serovars laboratory-confirmed human Salmonella infections in United States (CDC, 2013b).

In this study we cannot discern the underlying causes associated with longer shedding duration of serovars (e.g.; whether certain serovars may survive well in environment and therefore pigs are repeatedly exposed and/or if there is differential invasive/adaptive characteristics of the serovar in the host). Epidemiologic studies have demonstrated differential distribution of specific serovars and genotypes between pigs and farm environments (Farzan et al., 2008a; Dorr et al., 2009; Farzan et al., 2009), as for example S. Typhimurium was less likely to be isolated from the manure pit as compared to other serovars (Farzan et al., 2009). Moreover, experimental studies have shown certain serovars have a greater ability to establish infection, shedding patterns and concentration of Salmonella shed varies according to the serovar (van Winsen et al., 2001; Osterberg and Wallgren, 2008; Osterberg et al., 2009, 2010), suggesting that serovars better adapted to the pig may shed longer. These reports support that serovars have different ability to establish and maintain an infection. Actually, a modeling study of the referred data has shown Salmonella fecal shedding and pig immune response following the challenge are dose- and serotype-dependent (Ivanek et al., 2012).

Risk factors associated to serovar-specific shedding patterns should be further investigated. A European study reported S. Typhimurium and serovar 1,4,5,12:i:- were related to animal risk factors while environmental risk factors were associated with other serovars (Correia-Gomes et al., 2012). Identification of serovar-specific risk factors may allow implementation of control measures in order to preferentially reduce the serovars implicated in foodborne human illness.

Moreover, targeting certain serovars has become more important for those with an increasing public heath importance due to multidrug antibiotic resistance , and emerging serovars common isolated in swine, pork products and humans (Clothier et al., 2010; Hauser et al., 2010; Hauser et al., 2011; Keelara et al., 2013). Currently pre-harvest control measures are non-differential in terms of serovar; knowing the risk factors related to specific serovars will allow implementing control measures targeting those with greater risk to humans. Therefore, differential control measures based on serovars of public health importance might be more efficient and effective as opposed to the non-differential approach currently taken in swine production control schemes.

A significant difference was observed among sites despite belonging to the same production system and having an identical pig source, feed supply and overall management procedures. Variability in Salmonella prevalence among herds and within the same herd over time has been previously reported (Funk et al., 2001; Rajic et al., 2005; Pires et al., 2013a). The observed difference might be due to unmeasured factors associated with site, such as producer behaviors and biosecurity, as each site is under supervision of different personnel. Actually, the effect modifier of treatment status on site re-enforces that differences of management among sites may affect the shedding pattern of Salmonella infection in swine, either by reducing shedding as a result of antimicrobial effect or, an increased duration of shedding due to a selective resistance of Salmonella serovar-specific. The significant association between treatment and Salmonella shedding might also be a result of individual pig treatment being a proxy of overall cohort health and/or management practices. An association between Salmonella status and several swine diseases has been reported (van der Wolf et al., 2001; Beloeil et al., 2004; Beloeil et al., 2007). Nevertheless the limitation of treatment data being dependent on farmer recorded data and the number of treatments and not disease occurrence, we found that cohorts with treatment greater than the mean are more likely to shed Salmonella for shorter durations. The majority of the recorded treatments in this study were antibiotics. The underlying biological mechanism of interaction between treatment and Salmonella persistence is unclear ; on one hand the antibiotics might be responsible for disrupting the normal microbial flora and consequently increasing the colonization of the gastrointestinal tract by gram-negative bacteria such as Salmonella (Funk et al., 2007; Rajic et al., 2007). One the other hand, the use of antibiotics may have direct antimicrobial effects on the Salmonella. We do not know the antimicrobial resistance profiles of the isolates in this study.

There was a significant influence of nursery status (cohorts with positive nursery pools greater than the mean) on duration of fecal shedding. It seems that previous exposure during the nursery phase is associated with an increased duration of shedding. Pigs entering the finisher from these cohorts were exposed to Salmonella in the nursery and may have been shedding at arrival to the finishing barn. However, the nursery status was not associated with Salmonella serovar persistence in the more restricted data set that included pigs that shed only one unique serovar. A potential explanation for the difference between the two models is associated with the data structure. Pigs shedding the 3 major serovars where mainly from cohorts with a higher proportion of positive pools (107 pigs from nursery with positive pools greater than the mean, versus 20 pigs from cohorts with the number of pools less than the mean), therefore the two variables (nursery status and categorization of serovars) were highly correlated. Interestingly, the presence of same serovar in the nursery as found in the finishing phase was not significant. One of the explanations for the lack of significance could be due to a non-differential exposure among cohorts by losing information when this risk factor was categorized into a binary variable. In fact, the same serovar was isolated from both the nursery and individual pigs in a majority of cohorts (70.6%). These findings suggest that the shedding duration might be affected by the pathogen load prior to the pigs being moved to finishing barn, but whether the serovar of exposure is relevant is unclear since the data structure in this study may have hampered our ability to distinguish this potential risk.

One of limitations of this study is the inclusion of only one swine production company. One unique production company was selected in order to improve internal validity of the study, to control for potential confounders such as genetics, feed, treatment and vaccination protocols, biosecurity, and management practices. Nevertheless, the selected production system is representative of the swine industry in the US and the identified serovars are common to other North American studies. The results of this study warrant further studies to understand persistence of shedding between farms to

assess the external validity of these findings. The other limitation is related to sampling procedure and diagnostic tests. Because each pig was sampled with a 2 week of interval, pigs might be misclassified due to shedding between sampling times. Related to the diagnostic test, to some extent, culture sensitivity might differ for different serovars, and therefore the true relative prevalence of serovars might differ from those reported here (Funk, 2003; Love and Rostagno, 2008). In addition, pigs might be infected with more than one serovar (Funk, 2003) and in the present study only one colony per pig was selected for further serotyping. Despite being an imperfect diagnostic test, fecal culture is considered the 'gold standard' for Salmonella isolation (Funk, 2003) and the culture protocol commonly used in prevalence Salmonella studies in swine.

Producer Summary

Based on the results of this study, the duration of Salmonella shedding in pigs is associated with the Salmonella status of the nursery of origin (more positive samples from the nursery, the longer the duration of shedding), infection at a younger age (pigs first detected as positive at 10 weeks of age shed Salmonella longer than pigs 12 week of age or older at first positive Salmonella fecal sample) Salmonella serovar (S. Agona was shed for a longer duration) and the number of treatments in the group of pigs from which the pig originates (above average treatment rate decreased the duration of Salmonella shedding in swine). Identification of risk factors associated with duration of shedding may allow more targeted interventions to control Salmonella by evaluation of control measures not only for prevalence reduction, but also to decrease the duration of shedding once exposed. In addition, further evaluation of serovar targeted interventions, both from public health risk and on-farm control, is warranted.

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Tables:

Table 1: *Salmonella* serovar distribution in finishing pigs (187 pigs).

Serovar	Individual pig fecal samples (%) (N=446)	Nursery (%) (N=62)	Environment (%) (N=33)	Total
S. Derby	211 (47.3)	23 (37.1)	3 (9.1)	237
S. Agona	122 (27.4)	28 (45.2)	4 (12.1)	154
S. Johannesburg	47 (10.5)	3 (4.8)	9 (27.3)	59
S. Schwarzengrund	12 (2.7)	1 (1.6)	0	13
S. Litchfield	11 (2.5)	1 (1.6)	0	12
S. Mbandaka	10 (2.2)	0	0	10
S. Infantis	6 (1.3)	1 (1.6)	0	7
S. Livingstone	5 (1.1)	0	0	5
S. Bovis-morbificans	4 (0.9)	0	0	4
S. Meleagridis	4 (0.9)	1 (1.6)	0	5
S. Montevideo	4 (0.9)	2 (3.2)	17 (51.5)	23
S. Worthington	3 (0.7)	0	0	3
S. Typhimurium	2 (0.4)	0	0	2
S. Barranquilla	1 (0.2)	1 (1.6)	0	2
S. Lexington	1 (0.2)	0	0	1
S. Orion	1 (0.2)	0	0	1
S. Paratyphi B, L-tartrate+	1 (0.2)	0	0	1
S. Rough O:b:l,w	1 (0.2)	0	0	1
S. Muenchen	0	1 (1.6)	0	1

Table 2: Distribution of the number of events (clearance of *Salmonella*) by farm, pig age, sex , pig health status, nursery and environment *Salmonella* status and serovar, treatment and subject groups in a longitudinal study in finishing pigs where 175 pigs were included (151 events and 24 right-censored pigs).

Explanatory Variable	Level	N (%)
Farm	Site A	42 (27.81)
	Site B	70 (46.36)
	Site C	39 (25.83)
Pig age first detected	10 weeks	91 (60.27)
	Equal or greater than 12 weeks	60 (39.73)
Sex	Female	68 (45.03)
	Male	83 (54.97)
Pig health status[a]	Healthy	119 (78.81)
	Not healthy	32 (21.19)
Nursery *Salmonella* status[b]	Greater than mean	112 (74.17)
	Less than or equal to mean	39 (25.83)
Environment *Salmonella* status[c]	Positive	112 (74.17)
	Negative	39 (25.83)
Nursery serovar exposure[d]	Yes	132 (87.42)
	No	19 (12.58)
Environment serovar exposure[e]	Yes	92 (60.93)
	No	59 (30.07)
”At risk pig”[f]	Greater than mean	25 (16.56)
	Less than or equal than mean	126 (83.44)
Treatment[g]	Greater than mean	58 (38.41)
	Less than or equal than mean	93 (61.59)
Serovar	*S.* Derby	59 (39.07)
	S. Agona	33 (27.85)
	S. Johannesburg	18 (11.92)
	Others	41 (27.15)

Footnote (Table 2):

[a] Pig health status: Not healthy, when one of the events occurred at the sampling time:1) diarrhea; 2) sick or being moved to the sick pen; 3) undersized pig; 4) 'subject' pig; 5) any sign of disease observed by research personnel
[b] Nursery *Salmonella* status: overall mean of the total *Salmonella* positive pools (reference level: less than or equal to the mean 3.64 %)
[c] Environment *Salmonella* status: positive when at least one sample was *Salmonella* positive (reference level, all swabs were negative).
[d] Nursery serovar exposure: presence of the same serovar in nursery and respective cohort
[e] Environment serovar exposure: presence of the same serovar in environment swab samples and respective cohort
[f] ""At risk pig", subject median (reference level: less than or equal to the median, 1.87%) of proportion of total pigs that were defined by farm personnel as abnormal and housed separately, based on total of pigs placed at the beginning of each cohort
[g] Treatment: median of proportion of total individual treatments (reference: less than or equal to the median, 0.48%), based on total of pigs placed at the beginning of each cohort

Table 3: Results of the final acceleration failure time (AFT) model, with log-normal distribution, analysis of the time (days) for duration of *Salmonella* shedding, in 175 pigs in a longitudinal study in finishing pigs.

Explanatory variables	Level	Parameter estimate[c]	Overall p-value	Time ratio	95% Time ratio C.I.
Pig age first detected	10 weeks	0.46	0.007	1.59	1.13-2.22
	Equal or greater than 12 weeks	0		1	-
Nursery *Salmonella* status[a]	Greater than mean	0.53	<0.001	1.7	1.33-2.17
	Less than or equal to mean	0		1	-
Site	Site A	0.55	<0.001	1.73	1.50-2.0
	Site B	0.78	<0.001	2.19	1.77-2.71
	Site C	0			-
Treatment[b]	Greater than mean	0.11	0.27	1.12	0.92-1.37
	Less than or equal than mean	0		1	-
Interaction Site and Treatment	Site A and Treatment > than mean	-0.34	0.005	0.71	0.56-0.90

	Site B and Treatment > than mean	-0.85	<0.001	0.42	0.32-0.57
	Site C and Treatment <= than mean	0			
Sigma coefficient = 0.73 (95% C.I. 0.65-0.81)					

Footnote (Table 3):

[a] Nursery *Salmonella* status: overall mean of the total *Salmonella* positive pools (reference level: less than or equal to the mean 3.64 %)

[b] Treatment: median of proportion of total individual treatments (reference level: less than or equal to the median, 0.48%), based on total of pigs placed at the beginning of each cohort

[c] Parameter estimates with robust standard errors, accounting for clustering in 17 cohorts

Table 4: Results of the final acceleration failure time (AFT) model, with log-normal distribution, analysis of the time (days) for duration of the *Salmonella* shedding, in 151 pigs shedding one unique serovar[a] in a longitudinal study in finishing pigs.

Explanatory variables Variable	Level	Parameter estimate[c]	Overall p-value	Time ratio	95% Time ratio C.I.
Pig age first detected	10 weeks	0.47	0.018	1.6	1.2-2.1
	Equal or greater than 12 weeks	0		1	-
Treatment[b]	Greater than mean	-0.45	0.009	0.6	0.5-0.8
	Less than or equal than mean	0			-
Site	Site A	0.39	0.008	1.48	1.1-1.98
	Site B	0.68	0.004	1.98	1.23-3.16
	Site C	0			
Serovar[a]	S. Derby	-0.57	0.002	0.56	0.39-0.82
	S. Johannesburg	-0.7	< 0.001	0.49	0.36-0.68
	Others	-0.32	0.049	0.72	0.45-0.89
	S. Agona	0			-
sigma coefficient = 0.74 (95% CI 0.67-0.82)					

Footnote (Table 4):

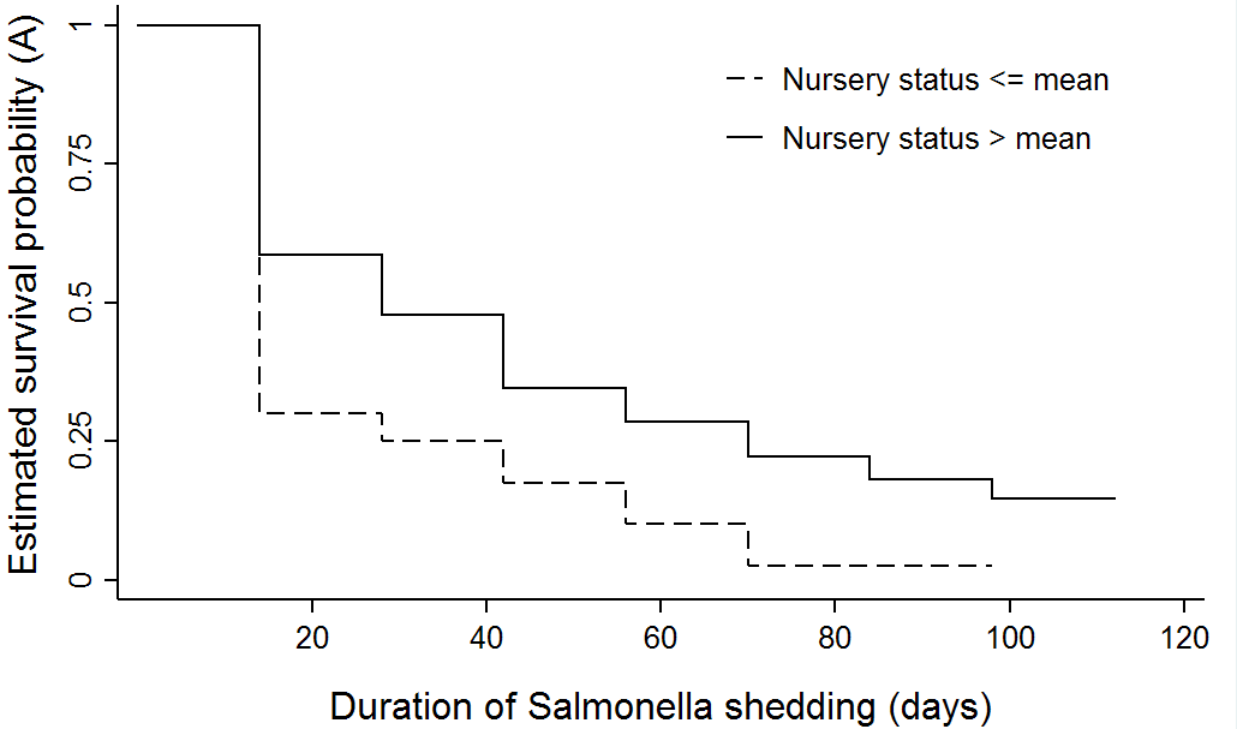
[a] *S.* Derby (66 pigs), *S.* Agona (41), *S.* Johannesburg (20), Others: *S.* Schwarzengrund (5), *S.* Litchfield (4), *S.* Mbandaka (5), *S.* Infantis (3), *S.* Bovis-morbificans (2), *S.* Meleagridis (2), *S.* Montevideo (1), *S.* Typhimurium (1), *S.* Paratyphi B, L-tartrate+ (1)
[b] Treatment: median of proportion of total individual treatments (reference level: less than or equal to the median, 0.48%), based on total of pigs placed at the beginning of each cohort
[c] Parameter estimates with robust standard errors, accounting for clustering in 16 cohorts

Table 5: PFGE Patterns by Serovar

Serovar	Number of isolates	Distinguishable PFGE patterns
S. Derby	39	16
S. Johannesburg	23	10
S. Meleagridis	2	2
S. Litchfield	2	2
S. Agona	34	13
S. Infantis	4	2

Figures:

Figure 1: Estimated survival probability plots (Kaplan-Meier survival curves) of time in days for *Salmonella* shedding stratified by nursery *Salmonella* status (A) (based on overall mean of the total *Salmonella* positive pools; reference level: less than or equal to the mean 3.64 %) and treatment (**B**) (median of proportion of total individual treatments based on total of pigs placed at the beginning of each cohort; reference level: less than or equal to the median, 0.48%).


Estimated survival probability (A)
0
0.25
0.5
0.75
1
20
40
60
80
100
120
Duration of Salmonella shedding (days)
Nursery status <= mean
Nursery status > mean

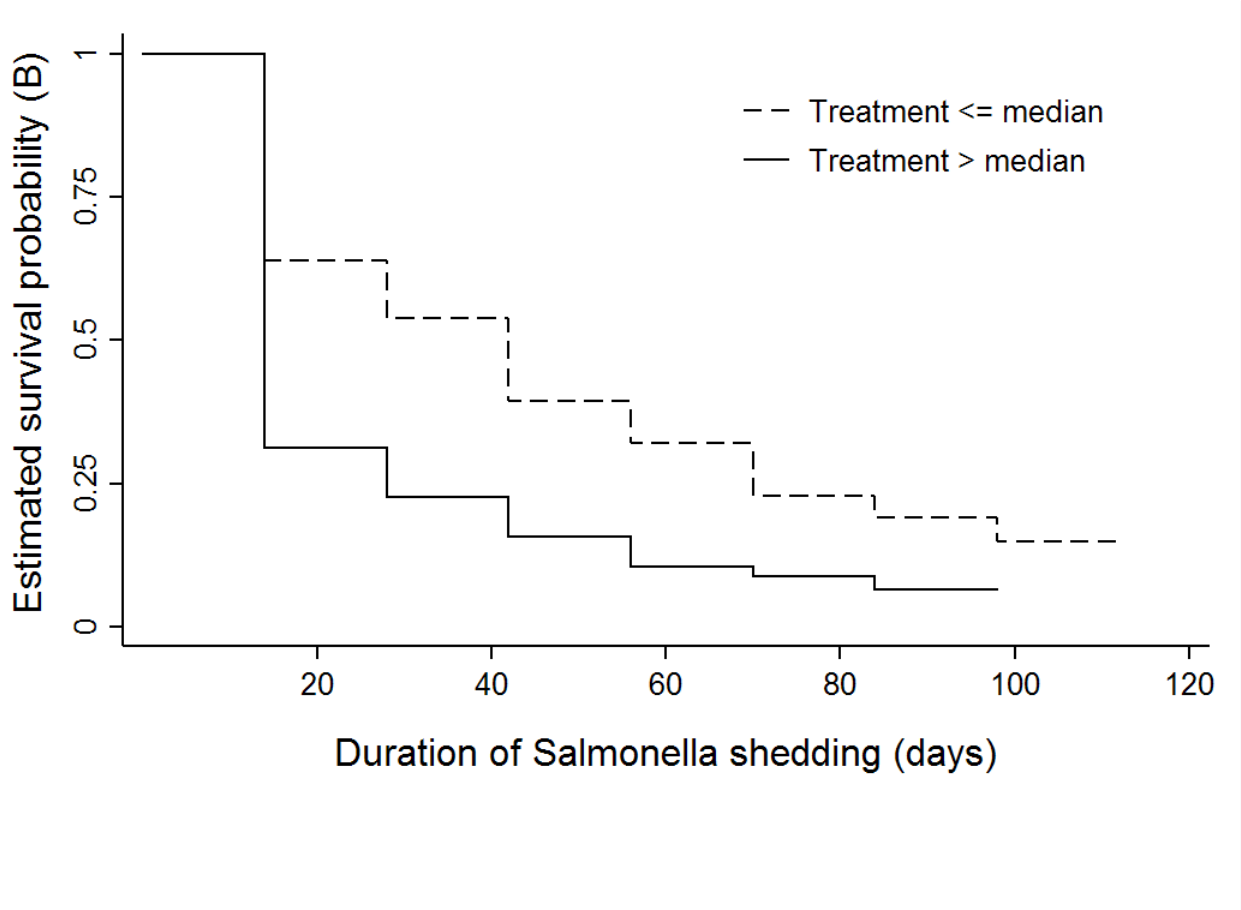
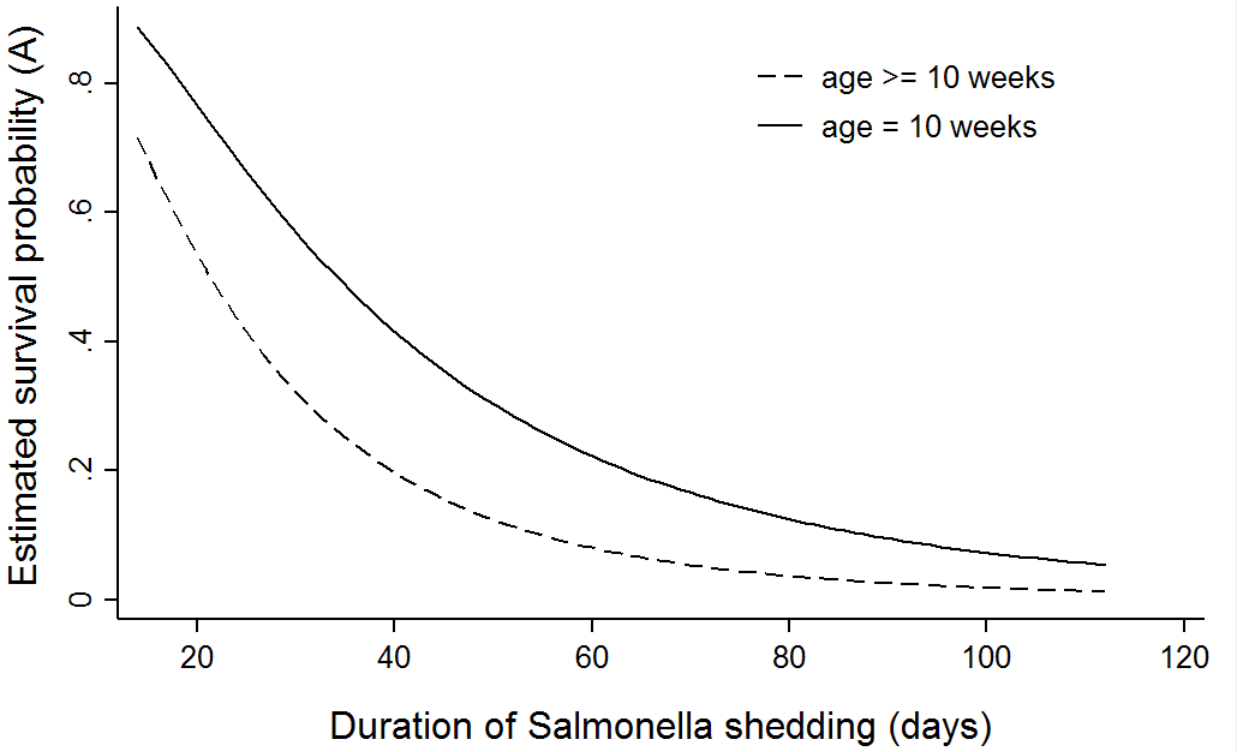
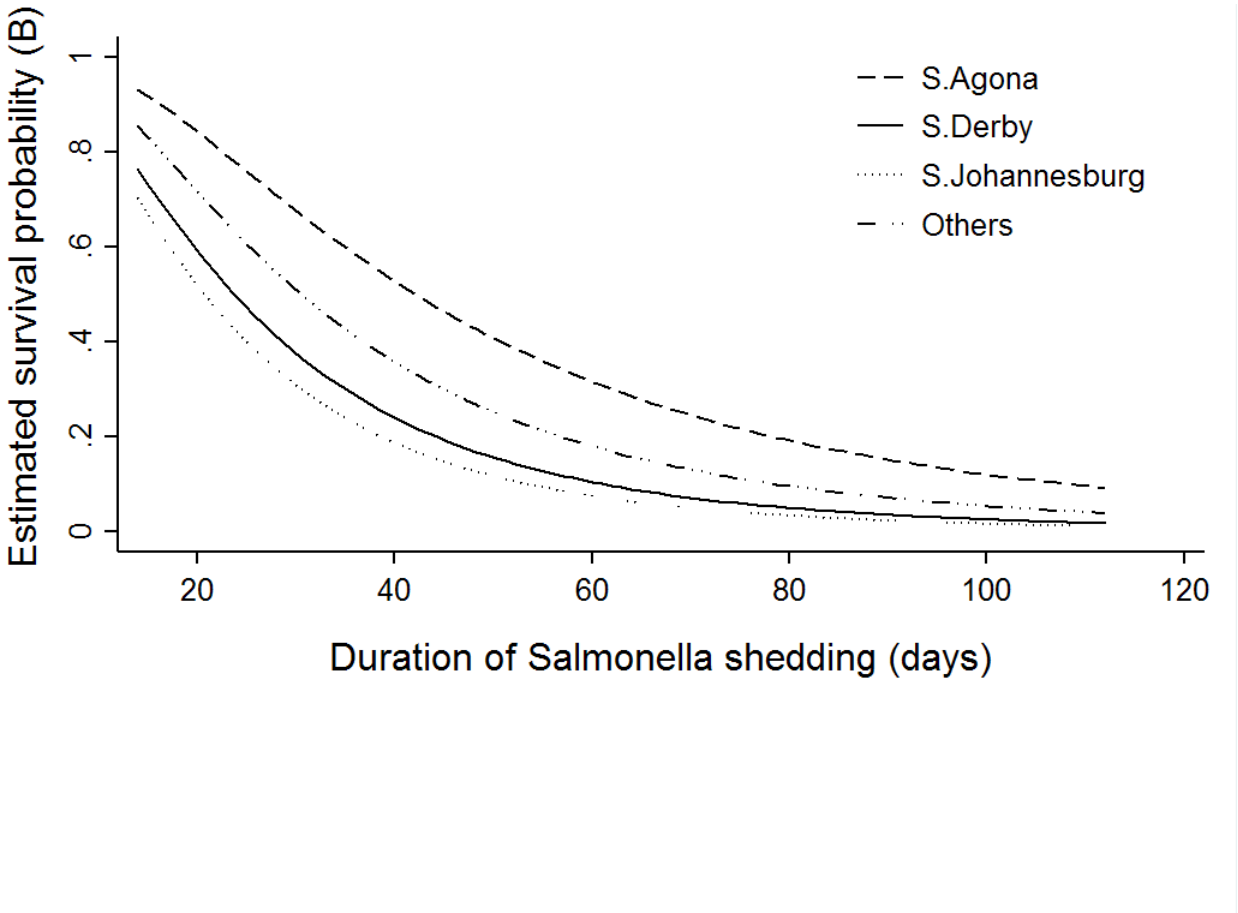


Figure 2: Estimated survival probability plots (acceleration failure time model) of time in days for *Salmonella* shedding infected with one unique serovar, stratified by age (pig age first detected positive) (A) and serovar (B) (*S.* Derby; *S.* Agona. *S.* Johannesburg and Others) of 151 pigs infected with one unique serovar [a].


Estimated survival probability (A)
0
.2
.4
.6
.8
20
40
60
80
100
120
Duration of Salmonella shedding (days)
age >= 10 weeks
age = 10 weeks



Footnote (**Figure 2**):

[a] *S.* Derby (66 pigs), *S.* Agona (41), *S.* Johannesburg (20), Others: *S.* Schwarzengrund (5), *S.* Litchfield (4), *S.* Mbandaka (5), *S.* Infantis (3), *S.* Bovis-morbificans (2), *S.* Meleagridis (2), *S.* Montevideo (1), *S.* Typhimurium (1), *S.* Paratyphi B, L-tartrate+ (1)